Ann. Anim. Sci., Vol. 17, No. 3 (2017) 757–770 *DOI: 10.1515/aoas-2016-0083*

EFFECT OF PRID-DELTA DEVICES ASSOCIATED WITH SHORTENED ESTRUS SYNCHRONIZATION PROTOCOLS ON ESTROUS RESPONSE AND FERTILITY IN DAIRY COWS

Irene López-Helguera[♦1,2], Fernando López-Gatius[1,3], Irina Garcia-Ispierto[1,2], Beatriz Serrano-Perez[1,2], Marcos G. Colazo[4]

[1]Agrotecnio Centre, University of Lleida, Lleida, Spain
[2]Department of Animal Science, University of Lleida, Lleida, Spain
[3]Transfer in Bovine Reproduction SLu, Barbastro, Spain
[4]Livestock Research Branch, Alberta Agriculture and Rural Development, Edmonton, AB, Canada
[♦]Corresponding author: irenelh@prodan.udl.cat

Abstract

This study evaluated the effect of a once-used progesterone (P4) intravaginal device (PRID) associated with four different shortened P4-based estrus synchronization (ES) protocols on estrous response (ER) and pregnancy per AI (P/AI) in cyclic and acyclic lactating dairy cows. Cows (n=465) were randomly assigned to one of the following protocols: 1) 2PGG, cows were given a PRID-Delta and 100 µg GnRH i.m. at PRID insertion (day 0). The PRID was left for 5 d, and 25 mg of dinoprost ($PGF_{2\alpha}$) i.m. given twice at PRID removal and 24 h later; 2) 2PGGe, same treatments as 2PGG plus 500 IU of eCG i.m. at PRID removal; 3) 2PGe, same treatments as 2PGGe, except GnRH was not given at PRID insertion; 4) PGe, same treatments as 2PGe, except PGF was only given at PRID removal. A total of 258 cows received a new PRID-Delta containing 1.55 g of P4, whereas 207 cows received a once-used PRID. Estrus was determined from P4 device removal until 96 h after using an automated heat detection system. Cows in estrus were given a second GnRH at AI and those without signs of estrus by 96 h after PRID removal were given GnRH and timed-AI (TAI). All inseminations were performed by one technician with commercially available frozen-thawed semen. Ultrasonography was performed at initiation of protocol and 28–34 days post AI to determine cyclicity and pregnancy status, respectively. Cows receiving once-used P4 devices had greater ER than cows receiving a new device (59.9 vs. 50.0; P=0.029), but P/AI did not differ between P4 devices, respectively (P>0.1; 40.6 vs 40.7%). Cyclic cows were less likely to display estrus than acyclic cows by a factor of 0.66 (P=0.036). Cows subjected to the 2PGe (2.41; P<0.01) protocol were more likely to display estrus than cows subjected to the 2PGG, whereas cows subjected to the PGe protocol did not differ (0.94; P=0.8) from those in the 2PGG group. Despite differences in ER, neither cyclicity nor estrus synchronization protocol affected P/AI (overall 40.6%). In summary, cyclic cows, those given a new P4 device and those subjected to either 2PGG or PGe protocol had reduced ER. However, all the factors examined had no significant effect on P/AI. All the estrus synchronization protocols resulted in acceptable fertility.

Key words: re-used PRID, acyclic dairy cows, estrus detection, pregnancy per AI

Although reproductive performance (measured as daughter pregnancy rate) in dairy cows has improved over the past decade, a high incidence of anestrus (Peter et al., 2009) and poor estrus detection (Roelofs et al., 2010) remain as major concerns in high producing dairy herds. These are cogent reasons why controlled breeding programs have become routine components of the reproductive management in dairy herds.

Induction of estrus and ovulation can be achieved with progesterone (P4) releasing intravaginal devices in combination with gonadotropin releasing hormone (GnRH) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Rhodes et al., 2003; Yániz et al., 2004; Macmillan, 2010; Colazo et al., 2013; Stevenson and Lamb, 2016). Moreover, these P4-based protocols allow for effective management of reproduction in high producing dairy cows, regardless of whether they are cyclic or acyclic (anestrus). Recently, shortened (5 d) P4-based protocol resulted in pregnancy per AI (P/AI) greater or similar to that obtained with longer protocols (Ribeiro et al., 2012; Garcia-Ispierto et al., 2013; Colazo and Ambrose, 2015; Muth-Spurlock et al., 2016; El-Tarabany, 2016). The initial GnRH treatment at P4 device insertion is intended to induce ovulation of a dominant follicle, and emergence of a new follicular wave within approximately 2 d (Martinez et al., 1999). Therefore, to ensure CL regression at device removal, two $PGF_{2\alpha}$ treatments are recommended in cows subjected to the shortened P4-based protocol (Kasimanickam et al., 2009). However, if the initial GnRH is not given, then a second treatment with PGF might be unnecessary (Colazo and Ambrose, 2015).

One of the anovulatory conditions most frequently described in high producing dairy cows is characterized by growth of follicles to larger than ovulatory size (Wiltbank et al., 2002). This condition is associated to an insensitivity of the hypothalamus to the positive feedback effects of estradiol (Wiltbank et al., 2002). There are also evidences that insufficient pulsatile release of LH is another reason for ovulatory failure in anestrus dairy cows (Peter et al., 2009). The equine chorionic gonadotropin (eCG), which has the capacity of binding to bovine LH and FSH receptors (Murphy and Martinuk, 1991), has been added to the timed-AI (TAI) protocols in particular in dairy herds with a high incidence of anestrus to increase the probability of ovulation after insemination (review in De Rensis and Lopez-Gatius, 2014). In this regard, confined lactating dairy cows with no estrus sign over 21 days subjected to a shortened P4 protocol that included one $PGF_{2\alpha}$ and eCG treatment resulted in P/AI similar to that obtained in cows that were AI after spontaneous estrus (Garcia--Ispierto et al., 2013). In another study, seasonally calving dairy cows diagnosed with anovulatory anestrus by rectal palpation treated with 400 IU of eCG at P4 device removal had increased 7-day (36.0 vs. 30.6%) and 28-day in calf rate (58.6 vs. 52.3%) compared to control (no eCG) anestrus cows (Bryan et al., 2013). In addition to the increased ovulation rate of healthier follicles, eCG could also enhance estrous behavior in acyclic cows increasing the service rate in those herds that utilize estrus detection (Garcia-Ispierto et al., 2012).

The P4 releasing intravaginal devices were originally designed for synchronization protocols with duration of 9 to 11 days (Review in Roche et al., 1992; Rhodes et al., 2003; Yániz et al., 2004; Macmillan, 2010). However, several studies have shown that once-used P4 devices resulted in P/AI comparable to those obtained with new devices in cows subjected to 7- to 9-d estrus and ovulation synchronization protocols (McPhee et al., 1983; Hanlon et al., 1997; Colazo et al., 2004). Therefore, it would plausible to speculate that the newly developed P4 device PRID-Delta could be used twice in shortened (5-d) protocols without negatively affecting its efficacy in inducing estrus and ovulation.

Thus, the overall objective of this study was to evaluate the effect of a once-used PRID-Delta associated with four different shortened P4-based estrus synchronization protocols on ER and P/AI in cyclic and acyclic lactating dairy cows. Our hypotheses were that a once-used PRID-Delta would result in ER and P/AI similar to that with a new device, whereas the addition of a second $PGF_{2\alpha}$ and eCG would further increase ER and P/AI in lactating dairy cows subjected to a shortened P4-based protocol.

Material and methods

Cattle and herd management

The present study was conducted in a commercial, high producing, Holstein-Friesian dairy herd in northeastern Spain. The herd comprised an average of 825 lactating cows during the study period between April 2013 and May 2014. Briefly, herd management entailed housing in free stalls with concrete slatted floors and cubicles, the use of fans and water sprinklers in the warm season (May to September), rigorous postpartum checks, confirmation of estrus at the time of artificial insemination (AI) by rectal palpation, and over 95% of AIs are done by veterinarians.

The mean annual culling rate was 26%. The mean annual milk production for the herd during the study period was 11,107 kg per cow. The cows were grouped according to age (primiparous and multiparous), milked three times daily and fed with total mixed rations. Dry cows were kept in a separate group and transferred to a “parturition group” 7 to 25 days before the expected parturition date, depending on their body condition score (López-Gatius et al., 2003; Roche et al., 2009) and whether or not they carried twins (López-Gatius and Garcia-Ispierto, 2010). An early postpartum, or “fresh cow”, group was established for postpartum daily checks and nutrition controls, and after 7 to 20 days postpartum, primiparous and multiparous lactating cows were transferred to two separate groups. Although estrus detection started on day 14 postpartum, the voluntary waiting period for the herd was 50 days.

Reproductive health management

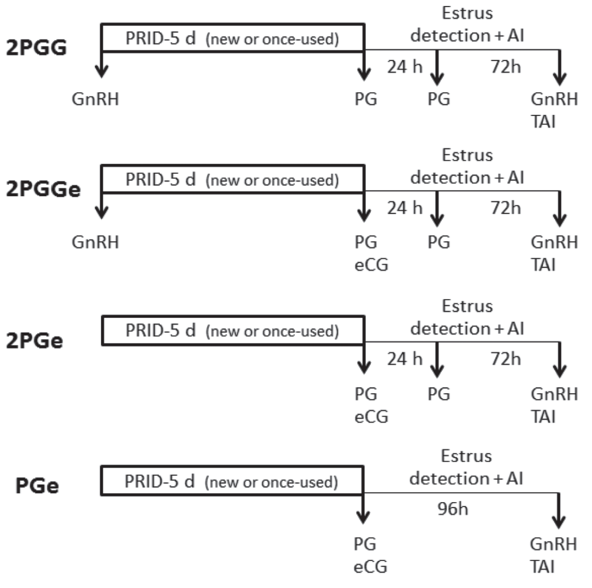
In the early postpartum daily checks, the following puerperal diseases were treated until resolved or until culling: signs of injury to the genital area (i.e., vaginal or recto-vulvar lacerations), metabolic diseases such as hypocalcemia and ketosis (for the latter, diagnosed during the first or second week postpartum), retained placenta (fetal membranes retained longer than 12 h after parturition), and/or puerperal metritis (diagnosed during the first or second week postpartum).

The herd was maintained on a weekly reproductive health program. This involved examining the reproductive tract of each animal by transrectal ultrasonography (Easi-scan with a 7.5 MHz transducer; BCF™ Technology Ltd, Livingston, UK) from 15 to 21 d postpartum to check for normal uterine involution and ovarian structures. Reproductive disorders diagnosed at this time such as endometritis or ovarian cysts were treated until resolved. Detectable cloudy intrauterine fluid was interpreted as endometritis (López-Helguera et al., 2012). An ovarian cyst was diagnosed when a follicular structure larger than 20 mm in diameter (external diameter including the wall) was detected in either or both ovaries in the absence of a corpus luteum (CL) and uterine tone (Hanzen et al., 2007). A second exam was performed close to the end of the voluntary waiting period (40 to 46 days postpartum) to re-check uterine and ovarian structures. Possible endometritis and/or ovarian cysts were also recorded and treated at this time. In the latter exam, a cow was recorded as suffering follicular anovulation when a follicular structure of at least 8-15 mm was detected in two consecutive examinations in the absence of a CL or cyst, and no estrus signs were noted during the 7-day period between the exams (López-Gatius et al., 2001).

Estrus synchronization protocols

During the weekly reproductive visit, 465 cows with more than 60 days in milk and at least 21 days with no estrus signs were randomly assigned to one of the following estrus synchronization protocols: 1) 2PGG, cows were treated with a progesterone (P4) releasing intravaginal device (PRID-Delta, containing 1.55 g of P4; CEVA Salud Animal, Barcelona, Spain) and GnRH (100 µg i.m.; Cystoreline, CEVA Santé Animale, Libourne, France) on day 0. The PRID was left for 5 d, and $PGF_{2\alpha}$ (25 mg dinoprost i.m.; Enzaprost, CEVA Santé Animale) given twice at PRID removal and 24 h later; 2) 2PGGe, same treatments as 2PGG plus 500 IU of eCG i.m. (Syncostim, CEVA Santé Animale) at PRID removal; 3) 2PGe, same treatments as 2PGGe, except GnRH was not given at PRID insertion; 4) PGe, same treatments as 2PGe, except PGF was given once at PRID removal. Cows in estrus, from P4 device removal until 96 h after, were given a second GnRH before AI and those without signs of estrus were given GnRH and TAI 96 h after PRID removal. All inseminations were performed by one technician with commercially available frozen-thawed semen. Illustration of activities and treatments during the study are shown in Figure 1.

Ovarian structures were assessed by ultrasonography on day 0. Cows with at least one CL were defined as cyclic, whereas cows without a CL and with no signs of estrus (i.e. uterine tone, uterine fluid) were considered as acyclic. Cows with ultrasound findings associated to estrus were inseminated and not included in the study. Cows were alternately assigned to receive a new or once-used PRID on a weekly rotational basis according to the chronological order of their gynecological visit. Thus, all once-used PRIDs were from cows synchronized during the previous week, and previously used for 5 days. Immediately after removal, PRIDs were washed thoroughly with soap and water for 4–5 min to remove accumulated mucus and debris, soaked for 2–3 sec in an iodine solution (0.05%), air-dried and stored for two days. Immediately previous to re-insertion, the devices were soaked again for 2–3 sec in the iodine solution.



Treatments:
PRID = Progesterone (P4) releasing intravaginal device containing 1.55 g of P4. GnRH=100 µg i.m.; $PGF_{2\alpha}$=25 mg dinoprost i.m.; eCG= 500 IU i.m.
Estrus was determined using an automated estrus detection system (Afimilk®) between Days 5 to 9. Cows were either AI at estrus or TAI 96 h after PRID removal. All cows received GnRH at AI.

Figure 1. Illustration of shortened P4-based protocols

Detection of estrus and pregnancy diagnosis

Estrus was determined using an automated estrus detection system (AfiFarm System; Afimilk®, Afikim, Israel). Walking activity values were recorded three times daily at the milking parlor and analyzed automatically using the herd management computer program. In a previous study (López-Gatius et al., 2005) using the same herd, an increased walking activity between 80 to 930% was significantly associated with fertility. Therefore, in the present study a walking activity value greater than 80% of the mean activity recorded in the previous two days was used as the minimum threshold for determining estrus (López-Gatius et al., 2005).

Estrus was confirmed by rectal palpation (López-Gatius and Camón-Urgel, 1988, 1991). Only cows with strong uterine contractility and copious transparent vaginal fluid were inseminated (López-Gatius, 2000). Cows that were detected in estrus in the afternoon and night milking were inseminated next morning, while cows detected in estrus in the morning were inseminated at noon. Cows that had an increased walking activity before the scheduled pregnancy diagnosis were examined by rectal palpation and if estrus was confirmed the animal was re-insminated and recorded as nonpregnant. In the remaining cows, pregnancy diagnosis was performed by transrectal ultrasonography 28–34 days post AI.

Cows diagnosed as nonpregnant were also examined for possible endometritis as previously described by López-Helguera et al. (2012). Cows with endometritis were treated and removed from the study.

Data collection and statistical analyses

The following data were recorded for each animal: parturition and treatment dates; parity (primiparous vs multiparous); estrus synchronization protocols (2PGG, 2PGGe, 2PGe or PGe); milk yield (mean production during the three days before initiation of protocol; low producers <40 kg versus high producers ≥40 kg); days in milk (DIM; <90 days postpartum vs ≥90 days postpartum); cyclicity (presence of at least one CL at initiation of protocol); type of PRID (new or once-used); ER after PRID removal (yes or not); sire; and pregnancy status 28-34 days post AI. In our geographical region, there are only two clearly differentiated weather periods: warm (May to September) and cool (October to April) (Labèrnia et al., 1998; Garcia-Ispierto et al., 2007). Treatment dates were used to analyze the effects of season on subsequent reproductive performance.

Two binary logistic regression analyses were performed. The dependent variables considered in these two analyses, respectively, were ER after PRID removal; and pregnancy 28–34 days post AI (P/AI). Season; parity; estrus synchronization protocols; milk production and days in milk at initiation of protocol; cyclicity at initiation of protocol; type of PRID; and sire were considered independent variables. For the dependent variable P/AI, ER was added as an independent variable. Regression analyses were conducted according to the method of Hosmer and Lemeshow (1989) using the logistic procedure of PASW Statistics for Windows Version 18.0 (SPSS Inc., Chicago, IL, USA). A probability of 0.05 or less was considered statistically significant, and a probability between 0.051 and 0.1 was considered a tendency.

Power analysis for the logistic regression was conducted using the G*Power 3.1 (Faul et al., 2007). Using an alpha of 0.05, a power of 0.90, and a two-tailed test, the desired sample size was 73, 275 and 180 for variables with odd ratios of 2.4, 1.5 and 0.6, respectively.

Results

The mean (± SD) values for milk production (kg) and number of lactation at the time of initiation of estrus synchronization protocols were 41.0±4.2 and 2.7±0.1, respectively. Of the 264 (56.8%) cyclic cows at the beginning of the synchronization protocol 69 (58.5%), 63 (57.8%), 65 (52.4%) and 67 (58.8%) cows were subjected to 2PGG, 2PGGe, 2PGe, and PGe, respectively. Two hundred and eighty and 185 cows were treated during cool and warm season, respectively. In total 258 cows received a new PRID, whereas 207 cows received a once-used PRID and 55.5% (207/465) of the cows had <90 DIM at initiation of the estrus synchronization protocol. No effect of milk production nor season was observed on either ER or P/AI. High producing cows (≥40 kg/day) had similar ER than lower producers [53.3% (188/353) vs

58.0% (65/112); P>0.1] as cows synchronized during warm or cool season [52.9% (148/280) vs 56.8% (105/185); P>0.1]. Moreover, P/AI in high and lower producers [37.9% (134/353) vs 49.1% (55/112); P>0.1] and in those synchronized during warm or cool season [38.4% (71/185) vs 42.1% (118/280); P>0.1] did not differ significantly.

Table 1 shows the variables included in the final logistic regression model for factors affecting the ER. Based on the odds ratios, cyclic cows at the initiation of protocol were less likely (by a factor of 0.66) to show estrus compared to acyclic cows (P=0.036). Cows receiving a once-used PRID were more likely (by a factor of 1.52) to show estrus than cows receiving a new PRID (P=0.001). Cows that did not receive GnRH at the beginning of protocol and were given two $PGF_{2\alpha}$ and eCG (2PGe group) were more likely (by a factor of 2.41) to show estrus than cows in the other protocols (P=0.001). Furthermore, ER was affected by an interaction between type of PRID and protocols. Although no differences were observed among protocols in cows given a once-used PRID, fewer cows given a new PRID showed estrus in 2PGG and PGe compared to 2PGGe and 2PGe protocols (Table 2).

Table 1. Odds ratios of the variables included in the final logistic regression model for factors affecting the estrous response (ER) (N=465)

Factor	Class	Cows in estrus, n (%)	Odds ratio	95% confidence interval	P-value
Cyclicity[1]	No	121/201 (60.2%)	Reference		
	Yes	132/264 (50.0%)	0.66	0.45–0.97	0.036
PRID	New	129/258 (50.0%)	Reference		
	Once-used	124/207 (59.9%)	1.52	1.04–2.23	0.029
ES protocols[2]	2PGG	55/118 (46.6%)	Reference		
	2PGGe	63/109 (57.8%)	1.53	0.90–2.61	0.113
	2PGe	84/124 (67.7%)	2.41	1.42–4.09	0.001
	PGe	51/114 (44.7%)	0.94	0.56–1.59	0.834

Hosmer and Lemeshow goodness-of-fit test = 0.403; 6 df, P=0.036.
R2 Nagelkerke = 0.072.
ER, cows in estrus (based on walking activity) from PRID removal to 96 h after removal.
[1]Cyclicity, based on the presence of at least one CL at initiation of protocol determined by transrectal ultrasonography.
[2]Estrus synchronization (ES) protocols:
2PGG: PRID for 5 d; GnRH on day 0; $PGF_{2\alpha}$ at PRID removal and 24 h later.
2PGGe: PRID for 5 d; GnRH on day 0; $PGF_{2\alpha}$ plus eCG at PRID removal and $PGF_{2\alpha}$ 24 h later.
2PGe: PRID for 5 d; $PGF_{2\alpha}$ plus eCG at PRID removal and $PGF_{2\alpha}$ 24 h later.
PGe: PRID for 5 d; $PGF_{2\alpha}$ plus eCG at PRID removal.
AI was performed at estrus or 96 h after PRID removal (TAI) in those cows not observed in estrus. All cows received a GnRH treatment at AI.

Endometritis was not diagnosed in any open cow at pregnancy diagnosis and the overall P/AI was 40.6% (189/465). Binary logistic regression analyses did not reveal significant association between the variables examined and P/AI. Cows receiving a new PRID had similar P/AI than those receiving a once-used PRID [40.7% (105/258) vs. 40.6% (84/207), respectively; P>0.1]. Cows subjected to 2PGG, 2PGGe, 2PGe and PGe had a P/AI of 46.6% (55/118), 45.0% (49/109), 36.3% (45/124) and 35.1%

(40/114), respectively (P>0.1). Similarly, P/AI between cows inseminated after estrus detection [43.5% (110/253)] and those TAI [37.3% (79/212)] did not differ (P>0.05; Table 2). No effect of DIM was observed on P/AI. In this regard, the P/AI in cows with <90 DIM at inititiation of the protocol was 43.0% (111/258) whereas P/AI was 37.7% (78/207) in those with ≥ 90 DIM.

Table 2. Effect of estrus synchronization protocols by type of P4 device on estrous response (ER) and by category of AI on pregnancy per AI (P/AI)

TAI Protocols[1]	2PGG (N=118) N (%)	2PGGe (N=109) N (%)	2PGe (N=124) N (%)	PGe (N=114) N (%)	Total (N=465) N (%)
Dependent variables[2]					
ER					
new PRID	26 (40.0) a	31 (58.5) b	49 (68.1) b	23 (33.8) a	129 (50) A
once-used PRID	29 (54.7)	32 (57.1)	35 (67.3)	28 (60.9)	124 (59.9) B
P/AI					
after estrus	30 (54.5)	30 (47.6)	31 (36.9)	19 (37.3)	110 (43.5)
after TAI	25 (39.7)	19 (41.3)	14 (35.0)	21 (33.3)	79 (37.3)

a, b – within a row and category, values without a common letters differed (P<0.05).
A, B – within a column and category, values without a common letters differed (P<0.05).
[1]Estrus synchronization protocols:
2PGG: PRID for 5 d; GnRH on day 0; $PGF_{2\alpha}$ at PRID removal and 24 h later.
2PGGe: PRID for 5 d; GnRH on day 0; $PGF_{2\alpha}$ plus eCG at PRID removal and $PGF_{2\alpha}$ 24 h later.
2PGe: PRID for 5 d; $PGF_{2\alpha}$ plus eCG at PRID removal and $PGF_{2\alpha}$ 24 h later.
PGe: PRID for 5 d; $PGF_{2\alpha}$ plus eCG at PRID removal.
AI was performed at estrus or 96 h after PRID removal (TAI) in those cows not observed in estrus. All cows received a GnRH treatment at AI.
[2]Dependent variables:
ER, cows in estrus (based on walking activity) from PRID removal to 96 h after removal; P/AI, pregnancy status diagnosed by transrectal ultrasonography 28–34 days post AI.

Table 3. Effect of estrus synchronization (ES) protocols on pregnancy per AI (P/AI) in cyclic and acyclic cows

ES protocol[1]	2PGG (N=118) N (%)	2PGGe (N=109) N (%)	2PGe (N=124) N (%)	PGe (N=114) N (%)	Total (N=465) N (%)
Acyclic	27 (55.1)	20 (43.5)	23 (39.0)	19 (40.4)	89 (44.3)
Cyclic	28 (40.6)	29 (46.0)	22 (33.8)	21 (31.3)	100 (37.9)

P/AI, pregnancy status diagnosed by transrectal ultrasonography 28-34 days post AI; Acyclic and cyclic cows, based on the presence of at least one CL at the initiation of protocol determined by transrectal ultrasonography.
[1]Estrus synchronization protocols:
2PGG: PRID for 5 d; GnRH on day 0; $PGF_{2\alpha}$ at PRID removal and 24 h later.
2PGGe: PRID for 5 d; GnRH on day 0; $PGF_{2\alpha}$ plus eCG at PRID removal and $PGF_{2\alpha}$ 24 h later.
2PGe: PRID for 5 d; $PGF_{2\alpha}$ plus eCG at PRID removal and $PGF_{2\alpha}$ 24 h later.
PGe: PRID for 5 d; $PGF_{2\alpha}$ plus eCG at PRID removal.
AI was performed at estrus or 96 h after PRID removal (TAI) in those cows not observed in estrus. All cows received a GnRH treatment at AI.

Albeit no significant difference was observed with regard to cyclicity, numerically more acyclic than cyclic cows (44.3 vs. 37.9%) become pregnant (Table 3).

Discussion

This study evaluated the estrous response and subsequent fertility in lactating dairy cows given a once-used or a new PRID in combination with four different shortened (5-day) synchronization protocols for AI.

Despite a single use is recommended by manufacturers, the reuse of P4 intravaginal devices result in a significant reduction in the cost of the estrus synchronization program. There is a lack of studies in the literature that have examined the residual P4 content in once-used PRIDs, which as new contain 1.55 g of P4. Rathbone et al. (2002) reported that the residual P4 content after a 7-day insertion period in a CIDR device was 0.72 g, which is approximately half of the original amount of 1.38 g. Although the amount of P4 remaining in a PRID-Delta after a 5 d treatment period is unknown, it is plausible to speculate that there is P4 enough to potentially be used at least twice in shortened estrus and ovulation synchronization protocols.

Administration of a once-used PRID-Delta increased ER before AI in the current study. The likelihood of ER was 1.5 times higher in cows receiving a previously used PRID. In agreement, McPhee et al. (1983) using a 9-day estrus synchronization protocol reported that 85% of cows, which received new PRIDs, came into estrus between 30 and 60 h after removal, whereas 100% of those receiving a once-used PRID displayed estrus within the same time. Interestingly, once-used autoclaved PRIDs or CIDRs maintained plasma progesterone profiles that were comparable to the release of P4 from new devices (McPhee et al., 1983; Zuluaga and Williams, 2008). However, this was not the case when PRIDs or CIDRs were re-used after disinfection by either gas sterilisation (McPhee et al., 1983) or soaking in a disinfectant solution (Zuluaga and Williams, 2008). Regardless of type of intravaginal P4 device, disinfestation with either method other than autoclaving might reduce the release of P4 following re-insertion. In this regard, an early study by McPhee et al. (1983) showed that mean plasma P4 in ovariectomized cows receiving a gas sterilized PRID were lower than that in those cows receiving a new device. Similarly, Zuluaga and Williams (2008) reported that mean serum concentrations (ng/mL) of P4 during a 7-d period were greater ($P<0.03$) for new (3.7 ± 0.2) than for once-used disinfected CIDR (2.8 ± 0.2). Once-used PRIDs utilized in the present study were soaked twice in an iodine solution right after first use and immediately before re-insertion. Although all previous studies have been performed with older devices and results cannot be directly extrapolated to newer devices made of different polymers such as the PRID-Delta device, differences in ER, in the present study, could have been associated to differences in concentration of circulating P4 between animals receiving a new device and those receiving a once-used device. It is well known that P4 regulates LH pulse frequency, which in terms, has a significant effect on follicle development and estradiol synthesis (Review in Inskeep, 2004). Therefore, we speculate that reduced P4 concentrations in cows given a once-used PRID might have increased LH concentrations, resulting in faster growth of the dominant follicles that also produced more estradiol (Cerri et al., 2011). In addition, larger follicles that were able to produce more estradiol right after PRID removal might have been associated to the greater proportion of animals displaying estrus in the once-used PRID group.

When the synchronization protocols were evaluated, our results indicate that the addition of a second $PGF_{2\alpha}$ treatment plus administration of eCG increased ER, partially supporting our original hypothesis. These differences were even more evident in cows receiving a new PRID. In a shortened synchronization protocol, if the initial GnRH treatment given at P4 device insertion induces ovulation of a dominant follicle, a young CL will be present at device removal. In order to ensure CL regression, two $PGF_{2\alpha}$ treatments are recommended in animals subjected to a shortened P4-based protocol (Kasimanickam et al., 2009). Therefore, it was not surprising that cows receiving a single injection of $PGF_{2\alpha}$ were less likely to display estrus after PRID removal, however, the lower ER observed in cows subjected to the 2PGG protocol was somewhat intriguing. Possibly the differences in ER among 2PGGe and 2PGe versus 2PGG protocol was due to only a slight delay in the manifestation of estrus and not to a poor luteal regression because fertility was not significantly reduced in cows in the 2PGG group. Cows in the 2PGe group did not receive GnRH at the initiation of the protocol, hence, one possibility was the formation of persistent follicles, which in turn would cause these cows to show estrus shortly after P4 device removal. However, this possibility contrasts the fact that the fertility was not compromised in these cows. This finding is in agreement with the observation that ovulation before TAI did not affect P/AI in dairy heifers subjected to a similar 5-d protocol (Colazo and Ambrose, 2011).

Administration of eCG at PRID removal might have further stimulated follicular growth and estradiol synthesis, due to its FSH and LH-like activity (Murphy and Martinuk, 1991), contributing to the increased ER observed in cows subjected to the 2PGGe or 2PGe protocols. Results from an early study (Bryan et al., 2013), involving anestrus seasonally calving anestrus dairy cows treated with a P4 device plus $PGF_{2\alpha}$ support our findings regarding the positive association between eCG treatment and ER.

Cyclic cows were less likely to display estrus than acyclic cows by a factor of 0.66. This is in agreement with previous results from our group. López-Gatius et al. (2015) observed that cyclic cows had lower ER after PRID removal compared to acyclic cows (46.2 vs 61.8%; P=0.03). The reasons for the increased ER after PRID removal in acyclic cows could not be determined in the present study. Acyclic cows, especially those that did not respond to initial GnRH, might have had lower plasma P4 concentrations during ovarian follicular development compared with that in cyclic cows. As discussed earlier, low concentrations of P4 have been associated with increased LH pulsatility and rate of growth of the ovulatory follicle. Therefore, acyclic cows might have larger and more mature preovulatory follicles at PRID removal that were able to trigger estrous behaviour sooner than cyclic cows.

Despite ER was significantly affected, none of the factors examined in this study had a significant effect on P/AI. The percentage of acyclic cows at the initiation of the synchronization protocol was relatively high (43.2%), but surprisingly, cyclicity did not affect P/AI. Moreover, numerically more acyclic cows compared to cyclic cows (44.3 vs. 37.9%) become pregnant following AI. Considering that more acyclic cows were inseminated after estrus detection, perhaps, the insemination strategy utilized in our study favored acyclic over cyclic cows. Overall P/AI did not differ between cows receiving a new device (40.7%) and those given a once-used device (40.6%), suggesting that PRID-Delta devices can be re-used without any negative effect on fertility. Several studies have shown that once-used P4 devices (i.e. PRID or CIDR) resulted in P/AI comparable to those obtained with new devices in cows subjected to 7- to 9-day estrus and ovulation synchronization protocols (McPhee et al., 1983; Hanlon et al., 1997; Colazo et al., 2004). Although the efficacy of cleaning and disinfection procedures utilized in this study is unknown, there were apparently no indications of disease transmission or infection associated with the re-use of PRID-Delta devices.

Although all the shortened P4-based estrus synchronization protocols utilized here have been previously developed for TAI, a combination of AI after a short period of estrus detection and AI was chosen in the present study. The overall P/AI was 46.6, 44.9, 36.3 and 35.1 for 2PGG, 2PGGe, 2PGe and PGe, respectively. Garcia-Ispierto and Lopez-Gatius (2014) compared the same protocols described here but cows were given a new PRID, GnRH 48 h after PRID removal and TAI 12 h later. In their study, P/AI ranged from 31.1 to 33.1%, which is lower than the overall 40.6% P/AI achieved in the present study. In this study timing of TAI was latter than usually performed, which might explain the numerically greater fertility in cows inseminated after estrus detection compared to those TAI. However, the combination with inseminations based on estrus detection might explain the overall P/AI observed in this study. Therefore, our results suggest that the inclusion of a short period of estrus detection would improve overall P/AI in lactating dairy cows subjected to shortened P4-based protocols.

Several studies have shown that the warm period of the year is one of the main factors affecting estrous behavior (López-Gatius et al., 2005) and fertility (López-Gatius, 2012) in lactating dairy cows in our geographical area. However, neither ER nor P/AI was affected by season in the present study. The administration of eCG to cows subjected to estrus synchronization protocols might overcome the negative effect of heat stress on fertility. In this regard, P/AI did not differ between lactating dairy cows inseminated after estrus detection during a cool period and cows subjected to a shortened P4-based protocol plus eCG for TAI during a warm period (Garcia-Ispierto et al., 2013). The success of shortened P4-based protocols is probably due to the ovulation of healthier oocytes from follicles with a shorter period of dominance and exposure to a longer proestrus (Bridges et al., 2008). Thus, we speculate that shortened P4-based protocols could overcome the negative effects of heat stress on estrus behavior and fertility in the current study.

In summary, cyclic cows, those given a new intravaginal P4 device and those subjected to either 2PGG or PGe protocols had reduced ER. However, cyclicity at initiation of protocol, type of intravaginal P4 device, ER and estrus synchronization protocol had no significant effect on P/AI. All the shortened AI protocols examined in this study resulted in acceptable fertility in acyclic and cyclic lactating dairy cows.

Acknowledgments

Authors thank the owner and the entire staff of Allué dairy herd (Catalonia, Spain) for their cooperation during the study.

References

Bridges G.A., Helser L.A., Grum D.E., Mussard M.L., Gasser C.L., Day M.L. (2008). Decreasing the interval between GnRH and PGF2a from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology, 69: 843–851.

Bryan M.A., Bo G.A., Mapletoft R.J., Emslie F.R. (2013). The use of equine chorionic gonadotropin in the treatment of anestrous dairy cows in gonadotropin-releasing hormone/progesterone protocols of 6 or 7 days. J. Dairy Sci., 96: 122–131.

Cerri R.L.A., Chebel R.C., Rivera F., Narciso C.D., Oliveira R.A., Amstalden M., Baez-Sandoval G.M., Oliveira L.J., Thatcher W.W., Santos J.E.P. (2011). Concentration of progesterone during the development of the ovulatory follicle: II. Ovarian and uterine responses. J. Dairy Sci., 94: 3352–3365.

Colazo M.G., Ambrose D.J. (2011). Neither duration of progesterone insert nor initial GnRH treatment affect pregnancy per timed-insemination in dairy heifers subjected to a Co-synch protocol. Theriogenology, 76: 578–588.

Colazo M.G., Ambrose D.J. (2015). Effect of initial GnRH and duration of progesterone insert treatment on the fertility of lactating dairy cows. Reprod. Domest. Anim., 50: 497–504.

Colazo M.G., Kastelic J.P., Whittaker P.R., Gavaga Q.A., Wilde R., Mapletoft R.J. (2004). Fertility in beef cattle given a new or previously used CIDR insert and estradiol, with or without progesterone. Anim. Reprod. Sci., 81: 25–34.

Colazo M.G., Dourey A., Rajamahendran R., Ambrose D.J. (2013). Progesterone supplementation before timed AI increased ovulation synchrony and pregnancy per AI, and supplementation after timed AI reduced pregnancy losses in lactating dairy cows. Theriogenology, 79: 833–841.

De Rensis F., López-Gatius F. (2014). Use of equine chorionic gonadotropin to control reproduction of the dairy cow: a review. Reprod. Domest. Anim., 49: 177–182.

El-Tarabany M.S. (2016). The efficiency of new CIDR and once-used CIDR to synchronize ovulation in primiparous and multiparous Holstein cows. Anim. Reprod. Sci., 173: 29–34.

Faul F., Erdfelder E., Lang A.G., Buchner A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods, 39: 175–191.

Garcia-Ispierto I., López-Gatius F. (2014). Effects of five-day progesterone-based fixed-time AI protocols on follicular/luteal dynamics and fertility in dairy cows. J. Reprod Dev., 60: 426–432.

García-Ispierto I., López-Gatius F., Bech-Sabat G., Santolaria P., Yániz J.L., Nogareda C., De Rensis F., López-Béjar M. (2007). Climate factors affecting conception rate of high producing dairy cows in northeastern Spain. Theriogenology, 67: 1379–1385.

Garcia-Ispierto I., López-Helguera I., Martino A., López-Gatius F. (2012). Reproductive performance of anoestrous high-producing dairy cows improved by adding equine chorionic gonadotrophin to a progesterone-based oestrous synchronizing protocol. Reprod Domest Anim., 47: 752–758.

Garcia-Ispierto I., Roselló M.A., De Rensis F., López-Gatius F. (2013). A five-day progesterone plus eCG-based fixed-time AI protocol improves fertility over spontaneous estrus in high-producing dairy cows under heat stress. J. Reprod. Dev., 59: 544–548.

Hanlon D.W., Williamson N.B., Steffert I.J., Witchell J.J., Pfeiffer D.U. (1997). Reinsertion of a progesterone-containing intravaginal device to synchronise returns to oestrus in dairy heifers. New Zeal. Vet. J., 45: 15–18.

Hanzen C.H., Bascon F., Theron L., López-Gatius F. (2007). Ovarian cysts in cattle Part 1: Definitions, symptoms and diagnostic. Ann. Med. Vet., 151: 247–256.

Hosmer D.W., Lemeshow S. (1989). Applied logistic regression. New York, John Wiley & Sons, 376 pp.

Inskeep E.K. (2004). Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. J. Anim. Sci., 82 E-Suppl: E24–39.

Kasimanickam R., Day M.L., Rudolph J.S., Hall J.B., Whittier W.D. (2009). Two doses of prostaglandin improve pregnancy rates to timed-AI in a 5-d progesterone-based synchronization protocol in beef cows. Theriogenology, 71: 762–767.

Labèrnia J., López-Gatius F., Santolaria P., Hanzen C., Laurent Y., Houtain J.Y. (1998). Influence of calving season on the interactions among reproductive disorders of dairy cows. Anim. Sci., 67: 387–393.
Larson L.L., Ball P.J.H., (1992). Regulation of estrous cycles in dairy cattle: A review. Theriogenology, 38: 255–267.
López-Gatius F. (2000). Site of semen deposition in cattle: a review. Theriogenology, 53: 1407–1414.
López-Gatius F. (2012). Factors of a noninfectious nature affecting fertility after artificial insemination in lactating dairy cows. A review. Theriogenology, 77: 1029–1041.
López-Gatius F., Camón-Urgel J. (1988). Increase in pregnancy rate in dairy cattle after preovulatory follicle palpation and deep cornual insemination. Theriogenology, 29: 1099–1103.
López-Gatius F., Camón-Urgel J. (1991). Confirmation of estrus rates by palpation per rectum of genital organs in normal repeat dairy cows. J. Vet. Med. Series A, 38: 553–556.
Lopez-Gatius F., Garcia-Ispierto I. (2010). Ultrasound and endocrine findings that help to assess the risk of late embryo/early foetal loss by non-infectious cause in dairy cattle. Reprod. Domest. Anim., 45(Suppl.3): 15–24.
López-Gatius F., Santolaria P., Yániz J., Rutllant J., López-Béjar M. (2001). Persistent ovarian follicles in dairy cows: a therapeutic approach. Theriogenology, 5: 649–659.
López-Gatius F., Yániz J., Madriles-Helm D. (2003). Effects of body condition score and score change on the reproductive performance of dairy cows: a meta-analysis. Theriogenology, 59: 801–812.
López-Gatius F., Santolaria P., Mundet I., Yániz J.L. (2005). Walking activity at estrus and subsequent fertility in dairy cows. Theriogenology, 63: 1419–1429.
López-Gatius F., Mirzaei A., Santolaria P., Bech-Sàbat G., Nogareda C., García-Ispierto I., Hanzen Ch., Yániz J.L. (2008). Factors affecting the response to the specific treatment of several forms of clinical anestrus in high producing dairy cows. Theriogenology, 69: 1095–103.
López-Gatius F., López-Helguera I., De Rensis F., Garcia-Ispierto I. (2015). Effects of different five-day progesterone-based synchronization protocols on the estrous response and follicular/luteal dynamics in dairy cows. J. Reprod. Dev., 61: 465–471.
López-Helguera I., López-Gatius F., Garcia-Ispierto I. (2012). The influence of genital tract status in postpartum period on the subsequent reproductive performance in high producing dairy cows. Theriogenology, 77: 1334–1342.
Macmillan K.L. (2010). Recent advances in the synchronization of estrus and ovulation in dairy cows. J. Reprod. Dev., 56: 42–47.
Martinez M.F., Adams G.P., Bergfelt D.R., Kastelic J.P., Mapletoft R.J. (1999). Effect of LH or GnRH on the dominant follicle of the first follicular wave in beef heifers. Anim. Reprod. Sci., 57: 23–33.
McPhee S.R., Doyle M.W., Davis I.F., Chamley W.A. (1983). Multiple use of progesterone releasing intravaginal devices for synchronization of oestrus and ovulation in cattle. Aus. Vet. J., 60: 40–44.
Murphy B.D., Martinuk S.D. (1991). Equine chorionic gonadotropin. Endocr. Rev., 12: 1305–1319.
Muth-Spurlock A.M., Poole D.H., Whisnant C.S., (2016). Comparison of pregnancy rates in beef cattle after a fixed time AI with once- or twice-used controlled internal drug release devices. Theriogenology, 85: 447–451.
Rathbone M.J., Bunt C.R., Ogle C.R., Burggraaf S., Macmillan K.L., Burke C.R., Pickering K.L. (2002). Reengineering of a commercially available bovine intravaginal insert (CIDR insert) containing progesterone. J. Contro. Release, 85: 105–115.
Rhodes F.M., McDougall S., Burke C.R., Verkerk G.A., Macmillan K.L. (2003). Invited review: Treatment of cows with an extended postpartum anestrous interval. J. Dairy Sci., 86: 1876–1894.
Ribeiro E.S., Monteiro A.P.A., Lima F.S., Ayres H., Bisinotto R.S., Favoreto M., Greco L.F., Marsola R.S., Thatcher W.W., Santos J.E.P. (2012). Effects of presynchronization and length of proestrus on fertility of grazing dairy cows subjected to a 5-day timed artificial insemination protocol. J. Dairy Sci., 95: 2513–2522.

Roche J.R., Friggens N.C., Kay J.K., Fisher M.W., Stafford K.J., Berry D.P. (2009). Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci., 92: 5769–5801.

Roelofs J., López-Gatius F., Hunter R.H.F., van Eerdenburg F.J.C.M., Hanzen C.H. (2010). When is a cow in estrus? Clinical and practical aspects. Theriogenology, 74: 327–344.

Sa Filho O.G., Dias C.C., Lamb G. C., Vasconcelos J.L.M. (2010) . Progesterone-based estrous synchronization protocols in non-suckled and suckled primiparous *Bos indicus* beef cows. Anim. Reprod. Sci., 119: 9–16.

Stevenson J.S., Lamb G.C. (2016). Contrasting effects of progesterone on fertility of dairy and beef cows. J. Dairy Sci., 99: 5951–5964.

Vos P.L.A.M., Ambrose D.J. (2009). Postpartum anestrus in dairy cattle. Theriogenology, 71: 1333–1342.

Wiltbank M.C., Gümen A., Sartori R. (2002). Physiological classification of anovulatory conditions in cattle. Theriogenology, 57: 21–52.

Yániz J.L., Murugavel K., López-Gatius F. (2004). Recent developments in oestrous synchronization of postpartum dairy cows with and without ovarian disorders. Reprod. Domest. Anim., 39: 86–93.

Zuluaga J.F., Williams J.L. (2008). High-pressure steam sterilization of previously used CIDR inserts enhances the magnitude of the acute increase on circulating progesterone after insertion in cows. Anim. Reprod. Sci., 107: 30–35.

Received: 21 IX 2016
Accepted: 20 XII 2016